PARTICLE ABSORPTION: SAMPLE FILTER PREPARATION AND ANALYSIS WITH AN INTEGRATNG SPHERE (IS)

Aimee Neeley
NASA GSFC, Greenbelt, MD
aimee.neeley@nasa.gov
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Supplies Needed

Combusted 25mm Whatman Glass fiber GF/F filters (0.7 μm nominal pore size)
Glass filter cups and stems
Gloves
Forceps
Histoprep tissue capsules (Fisherbrand 29x6 mm, Cat no: 15-182-219; white)
Kim wipes
Liquid nitrogen (LN) dewar

Prior to field campaign

- Combust the appropriate amount of 25mm GFF filters at 450°C for 6 hours. Note the lot number. During analysis you will use the same lot of filters.
- Combust the glass filter cups. Do not combust the stems; they will be damaged.

Sample Collection and Storage

- Set up **glass** filter cups/stems with **combusted** GF/F filters.
- Measure the required seawater volume with a graduated cylinder and pour into appropriate-sized amber bottle. **Record** filter volume (V_f) in log sheet.
- Filter samples under low vacuum (5-7 psi).
- Repeat steps 1 through 3 until appropriate amount of color can be seen on the filter. For particulate absorption, **LIGHTER IS BETTER**. Only filter enough to see lite color, other wise you may either overload the sample or make extraction of chlorophyll more difficult.
- When the appropriate volume has been filtered, rinse the filter cup with 0.2 um filtered seawater. Do not let filter run dry under vacuum. Close valve when last few milliliters (ml) are running through the filter.
- Remove filter from stem with forceps. Place filter into a CLEARLY pre-labeled histoprep and store in LN. Samples can be stored for a longer term at -80°C.

Sample Analysis

INSTRUMENT: Cary 100 UV-Visible scanning Spectrophotometer
S/N EL03127292

The Cary 100 is a double beam UV-Visible scanning spectrophotometer and is equipped with an integrating sphere (Labsphere DRA-CA-30, sold by Agilent Technologies: PN

190022900). It has a wavelength range of 190-900nm and a bandwidth range of 0.2 to 4nm.

Artificial Seawater (ASW) was used to maintain the moisture of the filters. The recipe was prepared using a modified version of Aquil Medium (Sunda et al., 2005). The recipe can found on the Provasoli-Guillard NCMA website under "Algal Medium Recipes." For this application, addition of the macronutrients, trace metals and vitamins is unnecessary.

a. Instrument Calibration and Maintenance

Instrument performance tests (wavelength accuracy and reproducibility, photometric noise, and baseline flatness) were conducted each day prior to analysis. Furthermore, National Institute of Standards and Technology (NIST)-traceable calibration standards (Holmium oxide filter for wavelength accuracy and Spectronics standards, Thermo Electron Corporation, to evaluate stray light, wavelength accuracy, and photometric performance) were also used to verify instrument performance.

b. Spectrophotometric Measurement Procedure

Scans were performed between 290-800nm with a 2nm Slit Band Width (SBW), 0.2nm data interval and 240nm per minute scan speed. The calculation for β was made using the equation from Rottgers and Gehnke (2012).

- Warm up the spectrophotometer for 30 minutes.
- Check the alignment of the beam and mirrors at ZERO order. The absorbance should be ~0.000 absorbance units. Adjust mirrors if this requirement is not met.
- Baseline the system with air. The air scan is performed to assess the stability of the system. The scan (instrument noise) should measure ~0.000 absorbance units ±0.005. If not, baseline the system again.
- Perform instrument performance tests.
- Place a blank GF/F filter moistened with ASW on the Plexiglas holder and jaw mount inside the integrating sphere chamber and scan. DO NOT baseline the instrument with the blank. The blank scan will be manually subtracted during data processing.
- Scan moistened blank filters (~3-5) periodically throughout the day to monitor instrument drift.
- For samples, place three to four drops of ASW into a petri dish. Place the sample filter biomass up onto the water droplet. Allow the sample filter to thaw for 5 minutes before measurement. Cover the petri dish with the lid and foil to protect from the light.
- Place the sample filter on the Plexiglas holder and jaw mount inside the integrating sphere chamber and scan at 0 and 90 degrees.

- Measure the diameter (D_f) of the biomass with calipers (Fisher Scientific Digital Caliper, model # 14-648-17)

Methanol Extraction method for de-pigmented particle absorption
The extraction protocol is based on Kishino et al. (1985).

- Place the sample filter on the glass filtration system.
- Gently add approximately 10-20ml of 95% methanol/5% ultrapure water to the filter cup and immediately filter at 5-7 psi.
- After the first 10-20 ml are filtered through, close the valve and add another 20 ml to the filter cup.
- Allow the sample to soak for AT LEAST 20 minutes. Cover the filter cup to prevent debris from contaminating the sample.
- Filter through and add another 20 ml methanol to the cup.
- Allow the sample to soak for at least another 20 minutes.
- After extraction, filter the last 20 ml of methanol through, and rinse the filter with 20 ml of ASW. DO NOT allow the filter to dry.
- Scan the moistened, extracted filter again using the protocol described in the *Spectrophotometric Measurement Procedure* section.
- If complete extraction of Chlorophyll is not attained, repeat the above extraction steps.

Data processing

- The average and standard deviation of the a_p and a_d scans were calculated.
- The mean of the blanks scans were subtracted across spectra from the mean a_p and a_d scans (OD_f).
- Absorption coefficient was calculated using the following equation

$a_p(\lambda) = [2.303*100/\beta*Pathlength]$

Pathlength = V_f (cm^3)/area of filter (cm^2)
Area of filter = $\pi*((D_f/10)/2))^2 = \pi r^2$
Diameter was divided by **10 to convert mm to cm and by 2 to get radius**
$\beta = 6.475*(OD_f \text{ ^2})-6.474*(OD_f)+4.765$ (Rottgers and Gehnke, 2012)

To calculate spectral absorption of phytoplankton:
$a_{ph}=a_p - a_d$

Data reporting

Each SeaBASS submission of a_p scans will include the following:

- Blank-corrected raw absorbance of both a_p and a_d
- Standard deviation of rotation scans for both a_p and a_d
- Absorption coefficient calculations for each replicate (where applicable) for a_p, a_d and a_{ph}

- Standard deviation of absorbance of all blank filters scanned throughout the analysis period
- Standard deviation of absorbance of air scans measured throughout the analysis period

Note: files that contain both replicates and more than one column of blank error indicates that replicates were analyzed on different days.

Reporting Notation

abs_ap = raw a_p with blank subtracted
abs_ap_sd = standard deviation of the filter rotations
abs_ad = raw a_d absorbance with blank subtracted
abs_ad_sd = standard deviation of the filter rotations
ap = absorption coefficient of total particles
ad = absorption coefficient of depigmented particles
aph = absorption coefficient of phytoplankton
abs_blank_sd = standard deviation of filter blanks
abs_air_sd = standard deviation of air scans

Kishino, M.N., Takahashi, N., Okami, N., and S. Ichimura, 1985. Estimation of the spectral absorption coefficients of phytoplankton in the sea. Bulletin of Marine Science. 37, 634-642.

Rottgers, R. and S. Gehnke, 2012. Measurement of light absorption by aquatic particles: improvement of the quantitative filter technique by use of an integrating sphere. Applied Optics. 51: 1336-1351.

Sunda, W.G., Price, N.M., and Morel, F.M.M., 2005. Trace metal ion buffers and their use in culture studies (Chapt. 4) pp. 35-63. In Andersen, R.A. (Ed.) *Algal Culturing Techniques*. Acad. Press/Elsevier, Amsterdam.

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